XXII. *On the Factors which determine the Cocoon Colour of* Plusia moneta *and other Lepidoptera.* By Mrs. Onèra A. Merritt Hawkes, B.Sc. (Lond.), M.Sc. (B'ham). Communicated by Dr. A. D. Imms, M.A.

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1. The Cocoon Colour of *Plusia moneta*.

The cocoons of *Plusia moneta* are normally bright yellow, but a white one is occasionally found. It is supposed by many entomologists that when such a phenomenon occurs, under natural conditions, in this or other species, that the whiteness is due to "weakness" of the larva. I have shown (Hawkes, 1916) that this is not the case in one Indo-Chinese hybrid moth (*Philosamia ricini* × *P. cynthia*), and it may be that further experiment will demonstrate that the lack of colour is usually due to well-defined environmental or physiological causes, rather than to any such indefinite condition as "weakness." Tutt in his "British Noctuae" only mentions yellow cocoons in *P. moneta*, but Barrett notes the variation from deep yellow to white.

In June 1916 two larvae of *Plusia moneta* were found feeding on Monkshood (*Delphinium*) in Edgbaston, Birmingham. They were reared in two glass-lidded boxes. When about to spin-up, all the leaves were removed from one box; thus, one box was dry, whilst the other was damp from the water evaporated from and transpired by the leaves. As a result, cocoons of two colours were spun—a yellow one in the damp box, and a white one in the dry box.

A piece of the white cocoon was then cut off and placed in the damp box, where, after twelve hours, it had become a deeper yellow than the complete yellow cocoon.

The larva in the yellow cocoon did not pupate, but, owing to some unknown cause, left its cocoon and died. The larva in the white cocoon pupated and produced a perfect imago. The white cocoon was kept in a dark dry box, the box being opened daily to admit fresh air. Towards the end of the pupal stage one end of the white cocoon had become yellowish, but it did not have even a vestige of the yellow lining described by Winser.

The experiment with the cut-off piece of silk makes it clear that an environmental cause, and not any excretion or secretion of the larva, was the *ultimate* producer of the yellow colour; the conditions under which the two larvae spun-up, and their subsequent history (it was the larva in the white cocoon which became an imago), indicates that environmental moisture and not "weakness" produced the difference in the cocoon colour. This conclusion is supported by the following independent observations.

1. J. H. Bird writes that empty white cocoons of *P. moneta* became yellow when immersed in water. R. S. Smallman, in discussing the remarks of Bird, also states that he believes moisture hastens the change from white to yellow, but in that case he would expect to find a larger proportion of white cocoons in the second brood, as that is produced in the late summer, when presumably there is less moisture than in the late spring; but, unfortunately, Smallman makes no statement concerning the relative numbers of the two colours in the two broods.

2. H. E. Winser states that most of his cocoons were white, but gives no indication as to whether the atmosphere in his breeding-cage was dry or damp; if the former, the white colour is accounted for.

3. Nicholson remarks that some have thought that light had an effect upon the colour.

It may here be stated that the two larvae under consideration in this paper were bred and spun-up in exactly the same conditions of light and heat, both breeding-boxes having been placed on a shelf in a large light room. The white cocoon which subsequently became yellowish at one end, did so at the same temperature, but in the dark.

4. Mr. W. H. Edwards of the Natural History Museum, Birmingham, was given, also in June 1916, two white cocoons of *P. moneta* which had been spun out of doors; these he put into his greenhouse, where in a couple of days they became yellow. The cocoons were spun during a period of drought, hence the white colour, and they became yellow when placed in the comparatively damp conservatory.

5. Sydney Webb and H. W. Andrews mention the variety of colour, and like Nicholson believe it is due to the amount

of light. H. W. Andrews found that cocoons on Larkspur were yellow, whilst those on Monkshood were white.

6. Bowles mentions that the cocoon, if spun indoors, is white, if out of doors, bright yellow.

The impression that white cocoons, such as the above, produce poor imagines, may be due to the deterioration of the pupa, if there is a continuance of the dry conditions under which such cocoons were spun. It is known by all breeders that the environmental condition during the pupal stage is a factor of great importance, and that very subtle differences in warmth and moisture may cause non-emergence or cripples. It is frequently more difficult to get the right environment for the pupa than for the larva. Crampton states, in reference to *Philosamia cynthia* (*advena*): "The perfect imagines constituted only 16·6% of the whole number of individuals which entered the cocoons, from which we may gain an idea of the severity of the conditions under which the quiescent pupa exists."

2. Experiments by various Observers upon White Cocoons of other Species.

Prof. E. B. Poulton has made extensive experiments with larvae which produce cocoons of various colours, and believes that in the majority of experiments white or pale cocoons were produced as a protective device by the larvae. If the white surroundings to which he refers (A) means white paper, which is dry when compared with green leaves, the whiteness may have been due to dryness.

One of his own experiments confirms my observations concerning the importance of a damp environment. On p. 450 (C) he relates that he put two larvae of *Halias prasinana* in two chip boxes (presumably dry), and both spun white cocoons. The first specimen, after having spun-up, was removed from its cocoon—in the process, it was cut and also found to be attacked by an ichneumon. It was then placed among oak leaves (comparatively damp), and there spun a brown cocoon. The second specimen was also removed from its cocoon, and placed in rolls of black net (presumably dry), where it began a second white cocoon. The second part of this experiment should be repeated, the black or any other coloured net being slightly damped.

Bateson in criticising the work of Poulton says, p. 205:

"The evidence which I brought forward went to show that the statement that there is any relation between the colour of these cocoons (*E. lanestris*) and that of the substance to which they are attached, was founded on a mistake"; and "it was to be concluded, that the cause determining the production of light cocoons was removal from the food, or the state of annoyance incidental to such removal, and that in fact the light-coloured cocoon was an abnormal product resulting from unhealthy conditions." This may well be the case in *Eriogaster*, but it is not the case in one *Philosamia* hybrid (loc. cit. p. 54), in *P. moneta*, and in the above two specimens of *Halias*.

Again, Bateson experimented with *Saturnia carpini*, and concluded (p. 207), "that there is no relation between the colour of the cocoons of *S. carpini* and that of the substances to which they are attached"; and (p. 209) "it may be safely concluded that the brown colour of the cocoons is derived from the alimentary canal." When Bateson placed pieces of white silk in the fluid ejected from the mouth of the larvae, the silk became brown; but Dewitz, who has also done experiments on *S. carpini* (= *S. pavonia*), found that when a piece of white silk was put in water it became brown.

May makes a report of two broods of larvae of *Saturnia carpini*. In brood 1, reared by Bell, the larvae were badly fed and kept in a practically air-tight cage with a damp atmosphere, the resulting sixteen cocoons being all dark. Brood 2 was reared by May, the larvae being well fed and kept in a dry, well-lighted cage; nineteen cocoons were produced—eighteen pale, one dark. When six of the pale ones came in contact with damp, they turned dark almost immediately. May says: "With regard to the one dark cocoon, it may have been splashed on pulling old stalks out of the water-bottle."

Dewitz believes that there is a "chromogene" in the silk of *S. carpini*, which under the influence of the oxygen of the air and an alkaline fluid becomes brown.

Comparing these results with my experiments on the *Philosamia* hybrid (p. 56), I am inclined to think that water *per se* can produce the change of colour in the completed silk.

Dewitz states that some hours after the last drop of frass has been expelled, a second, colourless intestinal excretion takes place in *S. carpini*, and that it is this that produces the brown colour. Dewitz and Bateson are in agreement as to an intestinal liquid producing the brown colour, but Bateson does not state that he distinguished between a first and a second ejection. Péligot, in one of the earliest papers on *Bombyx mori*, writes that the frass is first deposited, and sometime later a drop of clear alkaline liquid containing bicarbonate of potassium, and then "it (the larva) moves away and finds a place in which to spin." The second deposit in *Bombyx* differed from that of *S. carpini* in that it is deposited before the cocoon is spun, in which case it is difficult to see how it can have any subsequent influence on the silk.

Dewitz repudiates the statement of Levrat and Conte, that an intestinal fluid colours white silk by means of a foreign matter added to the outside of the silk fibres.

Dr. A. D. Imms kindly sent me two white cocoons of *Clisiocampa neustria*, which became yellow when placed in a damp box.

3. Results of the above Experiments.

So few attempts have been made to discover the ultimate cause of changes in cocoon colour, that the subject is almost untouched ground. The causes suggested for producing white cocoons may, *pro temp.*, be tabulated as follows. Each division is followed by the names of the moths of which we have any knowledge and the name of the observer.

I. The absence of an intestinal fluid (*i. e.* either an excretion from the intestine, or the secretion of the Malphigian tubules).

Saturnia carpini, Dewitz.
Poecilocampa populi, Tutt.
Hemaris fuciformis, Tutt.
Leucoma salicis, Tutt.
Eriogaster lanestris, Bateson.

II. A comparatively dry environment.

Plusia moneta, Merritt Hawkes.
Halias prasinana, Poulton (see p. 406 above).
Clisiocampa neustria, Merritt Hawkes.

III. A lack of foreign particles which are normally woven into the cocoon.

Hemerophila abruptaria, Poulton.
Chelonia caia, Tutt.

IV. A reduction of a yellow colour which is at first present.
Clisiocampa castrensis, Tutt.
C. neustria, Tutt.

This list by no means includes all the British moths which produce occasional white cocoons.

4. Mendelism and Cocoon Colour.

Kellogg in America and Toyama and his students in Japan have made a great number of breeding experiments with *Bombyx mori*, in order to study the inheritance of cocoon colour, but their results are not uniform, apparently because they dealt with different races. No such experiments have yet been undertaken with any British moths.

It is clear from the above observations that in the future all work done on cocoon colour, whether in studying heredity or environment, must take cognisance of the possible effect of moisture. In order to obtain reliable results, all cocoons must have *strictly* the same environment, not only as regards heat and light, but also as regards moisture. The lack of recognition of the response of cocoon colour to very small differences in moisture, has materially reduced the value of much work already done.

5. Conclusions.

1. Except as regards the addition of foreign particles, our knowledge of the causes of the change from white to brown or yellow silk is very superficial, and can only be made complete by a biological and chemical study of silk, both as a dead and a living product.

2. Even this superficial knowledge has yet to be extended to a considerable number of British species, and some experiments should be repeated, viz. those on *Saturnia carpini*.

3. Further investigation will probably co-ordinate the effect upon white silk of intestinal fluids and atmospheric moisture.

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